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HYPOGLYCAEMIC AND HAEMATINIC PROPERTIES OF ETHANOL LEAF EXTRACT OF *ARTOCARPUS HETEROPHYLLUS* IN ALLOXAN INDUCED DIABETIC RATS.

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Abstract

Background: Anaemia is known to be associated with diabetes; moreover, with the increasing cases of diabetes there is need for the use of more affordable alternative herbal medicines for the treatment of diabetes and anaemia. The aim of this work was to evaluate the hypoglycaemic and haematinic properties of *Artocarpus heteropyllus* on diabetic rats.
Materials and Methods: Ethanol leaf extract of *Artocarpus heteropyllus* was screened for phytochemicals and its acute toxicity was tested on mice. Induction of diabetes was done at a dose of 150 mg/kg body weight (b.w) (with exception of the control group). The extract was administered to rats for a period of 7 days at 100, 300 and 500 mg/kg b.w, respectively, following induction. Blood samples of rats were tested for fasting blood sugar (FBS), packed cell volume (PCV), white blood cell (WBC), red blood cell (RBC), haemoglobin, neutrophil lymphocyte and eosinophil counts.
Results: The ethanol leaf extract of *A. heterophyllus* showed no mortality up to a dose of 5000 mg/kg b.w. Administration of the extract to diabetic rats resulted in a decrease in the FBS of diabetic rat, and significant increases ($p< 0.05$) in RBC, PCV, WBC and haemoglobin levels.
Conclusion: The ethanol leaf extract of *A. heterophyllus* increased the haematological indices of diabetic rats. Our findings support the use of this plant as an herbal alternative in the treatment of diabetes and anaemia associated diabetes.

Key words: Diabetes, Hypoglycaemia, Haematology, Alloxan, Oxidative stress.

Introduction

There has been an increasing cases of diabetes across the globe owing to increase in population, aging, physical inactivity and increasing prevalence of obesity (Wild et al., 2004). Diabetes mellitus is a hereditary, metabolic disease characterized by hyperglycaemia with eventual polyuria (Brown, 2001). It is also characterized by the inability of tissues to carry out normal metabolism on carbohydrates, fats and proteins, due to an absolute or relative lack of insulin. The primary feature of this disorder is elevation in blood glucose levels (hyperglycaemia), resulting from either a defect in insulin secretion from the pancreas, a change in insulin action, or both (Bucirelli *et al.*, 2002). Sustained hyperglycaemia affects almost all tissues in the body and is associated with significant complications on multiple organ system, including the eyes, nerves, kidneys, and blood vessels (Naka et al., 2004).

Significant approaches have been made in the treatment and management of diabetes, although some orthodox treatment options are expensive and are not affordable by the lower class people in Africa. It is therefore necessary to find treatment and cure for diabetes and anaemia (resulting from diabetes) through the use of plants and plant derived products that are much more affordable and less expensive to orthodox medicine. More than 12 % of diabetes is associated with anaemia (Rani *et al.*, 2010), and diabetic anaemia if untreated can result in more severe conditions such as diabetic retinopathy and nephropathy (Singh et al., 2009; Stevens *et al.*, 2003). This study involved the use of local herbs in the treatment of diabetes and anaemia associated diabetic mellitus. Therefore, the present study evaluated the effect of ethanol leaf extract of *A. heterophyllus* on the hypoglycaemic and haematological indices of alloxan induced diabetes in rats.

A. heterophyllus belong to the mulberry family (Moraceae) and is known by other names such as Jackfruit in English, Kathal and Panas in Hindus, Kanthal in Bengal, Palaa in Tamil. The flakes of the ripe fruits of *A. heterophyllus* are rich in nutritive value; every 100 g of ripe flakes contains 287-323 mg potassium, 30.0-73.2 mg calcium and 11-19 g carbohydrates (Elevitch and Manner, 2006). The nutritious seeds are boiled or roasted and eaten like chestnuts, added to flour for baking, or cooked in dishes (Arung *et al.*, 2006). The shoots of *A. heterophyllus* have been shown to possess some nematocidal activity against various nematodes *viz.*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, *Tylenchus filifofmis* and *Meloidogyne incognita* (Sharma and Trivedi, 1995). The leaves and roots have been used for medicinal purposes. The crude methanol extracts of the stem, root, barks, leaves, fruits and seeds of *A. heterophyllus* and their subsequent partitioning with petrol, dichloromethane, ethyl acetate and butanol gave fractions that exhibited a broad spectrum of antibacterial activity. The butanol fractions of the root bark and fruits were found to be the most active (Khan et al., 2003). The anti-inflammatory effects of the isolated compounds were evaluated by determining their inhibitory effect on the production of pro-inflammatory mediators in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells (Rahman et al., 1999).

Materials and Methods

Materials

Collection and identification of plant materials

The leaves of *A. heterophyllus* were collected from Umunko in Igbo Etiti Local Government Area, Enugu State, Nigeria. The plant was identified by Mr. A. Ozioko of Bio-resources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State, Nigeria and a specimen of the plant was deposited at BDCP with voucher number BR 768. The fresh leaves of *A. heterophyllus* were first washed with distilled water and subsequently with normal saline to remove dirt and possible mycotoxins. The samples were dried under shade for several days and then pulverized into fine powder.

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Preparation of ethanol leaf extract of *A. heterophyllus*

The dried leaves of *A. heterophyllus* were pulverized to powder with a hand Mill. About 400 g of the pulverized leaves were macerated in 1.2 L of ethanol with thorough shaking at regular intervals for 48 h at room temperature (28 ± 2 °C). The resulting solution was then filtered using Whatman No. 4 filter paper. The filtrate was concentrated to a semi-solid residue using a rotary evaporator.

Phytochemical screening

The phytochemical analysis of the extract was carried out according to the methods of Harborne (1973); Trease and Evans (1989) to identify its bioactive constituents.

Acute toxicity (LD50) test

The method of Lorke (1983) was used for the acute toxicity test. Thirteen (13) albino mice were utilized in this study. The test involved two stages. In stage one; the rats were grouped into three (3) different groups of three rats each. They were administered 10, 100 and 1000 mg/kg body weight, respectively and in the second stage, 1600, 2900 and 5000 mg/kg body weight of the extract were administered to the rats. The administration of the extract was done orally. The ethanol leaf extract of *A. heterophyllus* was found not to be toxic up to 5000 mg/kg (Table 3); which indicates that the plant extract is safe for consumption.

Experimental design and induction of diabetes with alloxan

Thirty five (35) Wistar albino rats weighing between 102 and 240g were used for the study and were obtained from the Rat house of the Department of Zoology, University of Nigeria Nsukka. They were acclimatized to laboratory conditions for one week before the commencement of the study. They were fed *ad libitum* on standard pellet feed (Grand Cereals Ltd, Enugu, Nigeria) and freely provided drinking water. The baseline blood glucose levels were determined before diabetes induction The rats were fasted overnight prior to intraperitoneal injection of alloxan dissolved in iced cold normal saline at a dose of 150 mg/kg b.w. The blood glucose levels of the rats were determined after 3 days of induction and those with blood glucose level greater than 200 mg/dL were considered diabetic (Kumar *et al*., 2007; Frode and Medeiro, 2008) and were used in this study. The rats were divided into seven groups (n = 5). Diabetes was induced in rats from groups 2 through 6 and no induction was done in rats in groups 1 and 7. After induction, rats in group 1 received only normal saline, those in group 2 received 2.5 mg/kg b.w of Glibenclamide (standard control), those in group 3 received only normal saline (diabetic control), while those in groups 4, 5 and 6 received respectively 100, 300 and 500 mg/kg b.w of the plant extract. Group 7 rats were not induced with diabetes and received 500 mg/kg b.w of extract. The extract was administered to the rats through oral intubation. At the end of 7 days blood samples were drawn from the rats through ocular puncture and were received into clean EDTA tubes for haematology. The following haematological parameters assayed were packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and differential white blood cell counts were assayed after 7 days of treatment with the extract using the methods described by Ochei and Kolhatkar (2008).

Statistical Analysis

Data were reported as mean±SD. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 19. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean ± standard deviation. $P < 0.05$ was considered significant.

Results

Qualitative phytochemical screening

The qualitative phytochemical screening shows that *A. heterophyllus* contains alkaloids, flavonoids, glycosides, proteins, carbohydrates, saponins, tannins, terpenoids and steroids (Table 1).

Table 1: Preliminary Qualitative Phytochemical Screening of ethanol leaf extract of *A. heterophyllus*

phytochemicals		Phytochemicals		Phytochemicals	
Alkaloids	++	Proteins	+++	Tannins	+++
Flavonoids	+	Carbohydrates	+++	Terpenoids	++
Glycosides	+	Saponins	+	Steroids	++

+++ present in high amount, ++ present in moderate amount, + present in low amount, - absent

Effects of the Extract on fasting blood sugar (FBS)

The rats in the diabetic control group after 7 days of treatment still had a higher blood glucose level (glucose concentration after treatment) which was significant ($p<0.05$) when compared to the glucose concentration after induction, an indication that the rats were still in diabetic state. There were significant ($p<0.05$) decreases in the fasting blood sugars of rats in the treatment groups and in those of the standard control when compared to the diabetic control. The decreases observed in the fasting blood sugars of rats for each group were not significant ($p>0.05$) when the glucose concentrations after treatment is compared to those after induction of diabetes within each group (Fig 1).

Effect of the extract on haematological indices

A significant ($p<0.05$) increase were observed in the packed cell volume (PCV) of the rats in the treatment groups that received the extract of *A. heterophyllus* extract compared with those in the diabetic control group. The rats in the group that received no induction but fed 500 mg/kg b.w of the plant extract had a higher PCV (44.00±0.5) compared with those of the diabetic (38.33±1.20), standard (39.00±1.67) and normal (39.33±1.67) controls (see Table 3).

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The haemoglobin concentrations of the treatment group equally increased in a similar pattern as those of the PCV. Significant increase ($p<0.05$) were observed in the haemoglobin concentrations of the rats in the groups treated with 100 and 500 mg/kg b. w of the extract. The increase in haemoglobin observed in the rats fed 300 mg/kg b.w was not significant ($p>0.05$) (see Table 3). A significant ($p<0.05$) increase in RBC of rats in all the treatment groups was observed when compared to the rats in the control groups (diabetic, normal and standard controls) with exception to those that received 100 mg/kg extract.

Table 2: Results of the acute toxicity (LD_{50}) of the extract of *A. heterophyllus*

Phases 1	Dosages mg/kg body weight	Mortality
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phases 11		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

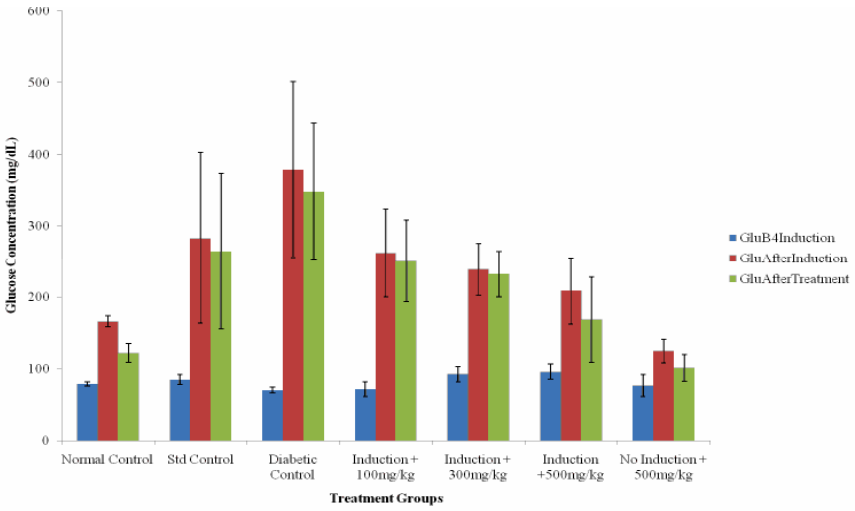

Figure 1: A graph showing the glucose level of alloxan induced diabetic rats before and after the induction of the extract and after treatment with Glibenclamide.

Significant ($p<0.05$) decreases were observed in the white blood cell (WBC) counts of all the rats in the groups that received the plant extract when compared to the standard and normal controls; but when the WBC counts of the treatment groups are compared to the diabetic control, significant increases ($p<0.05$) were observed. No significant differences ($p>0.05$) were observed in the neutrophil counts of all the rats that received the plant extract when compared with those in the diabetic and standard control groups; but a significant decrease ($p<0.05$) was observed in the rats that received 300 mg/kg b.w with those in the group that had no induction but received 500 mg/kg b.w of the plant extract when compared with the normal control. No significant ($p>0.05$) differences were observed in the lymphocyte counts of all the treatment groups compared to the diabetic and standard control groups; but significant increase ($p<0.05$) were observed when compared with the normal control. There were significant ($p<0.05$) increase in the eosinophil counts of all the rats in the treatment groups compared with the standard control.

Table 3: The haematological profile of diabetic rats treated with ethanol leaf extract of *A. heterophyllus* and those of the controls

	Normal Control	**Std Control**	**Diabetic Control**	**Induction + 100 mg/kg**	**Induction + 300 mg/kg**	**Induction + 500 mg/kg**	**No Induction + 500 mg/kg**
PCV (%)	39.33±1.67	39.00±1.15	38.33±1.20	42.33±1.45^{abc}	42.33±0.85^{abc}	40.67±0.33^{c}	44.00±0.58^{abc}
Hb (mg/dL)	13.00±0.33	12.67±0.88	12.80±0.17	14.33±0.33^{ac}	13.67±0.88^{b}	13.67±0.67^{c}	14.33±0.58^{abc}
RBC (x10^2/mL)	276.67±12.09	271.07±36.56	284.00±8.72	270.00±5.77^{c}	316.67±8.82^{abc}	336.67±8.82^{ab}	343.33±3.33^{ab}
WBC (mm^3)	8533.30±352.76	11400.00±503.32	3450.00±259.81	5500.00±57.74^{abc}	5966.00±202.76^{bc}	6400.00±57.74^{bc}	5200.00±115.47^{abc}

Values with the superscripts *a, b or c* indicates that there are significant differences compared to those of the normal, standard or diabetic controls respectively for each of the parameters at $p<0.05$.

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Table 4: The differential white blood count profile of diabetic rats treated with ethanol leaf extract of *A. heterophyllus* and those of the controls

	Normal Control	Std Control	Diabetic Control	Induction + 100 mg/kg	Induction + 300 mg/kg	Induction + 500 mg/kg	No Induction + 500 mg/kg
Neutrophil (%)	73.33±2.91	67.33±4.81	70.00±1.15	68.00±3.06	65.33±3.53 ^{a}	69.00±0.58	64.00±1.15 ^{a}
lymphocyte (%)	25.33±2.40	32.00±5.03	29.33±0.67	30.67±2.91 ^{a}	33.33±2.91 ^{a}	30.00±0.00 ^{a}	34.00±1.15 ^{a}
Eosinophils (%)	1.33±0.67	0.67±0.67	1.33±0.67	1.33±0.67 ^{b}	1.33±0.67 ^{b}	1.33±0.67 ^{b}	2.00±0.67 ^{abc}

Values with the superscripts *a, b or c* indicates that there are significant differences compared to those of the normal, standard or diabetic controls respectively for each of the parameters at $p<0.05$.

Discussion

Diabetes mellitus is the most important and common heterogeneous metabolic syndrome involving the endocrine organ, the pancreas (Frode and Medeiro, 2008), and is projected to be one of the world major killers in the near future (Naka et al., 2004). Many breakthroughs have been achieved in the treatment of diabetes; the most important being the production of recombinant insulin. Other treatment options of diabetes are expensive and most times cannot be afforded by the poor. Therefore, the need to screen local herbs for their hypoglycaemic activities become pertinent. The use of alloxan in diabetes induction in rats has been described as a useful experimental model for studying the effect of hypoglycaemic agents (Kar et al., 2003; Meral et al., 2004; Kumar et al., 2007; Ibrahim and Abdalla, 2011). Alloxan and its reductive product, dialuric acid, initiate a redox sequence resulting in the production of superoxide radicals. Superoxide radicals are reduced to hydrogen peroxide with a corresponding increase in cytosolic calcium ions which in concert with the radicals results in the destruction of the β cells of the pancreas (Brown, 2001). Destruction of the β cells of the pancreas result in diabetes.

Following induction with alloxan, a significant ($p<0.05$) increase in the serum glucose levels of the rats were observed and this establishes a state of hyperglycaemia. Treatments with ethanol leaf extract of *A. heterophyllus* resulted in decrease in the FBS of the rats in all the experimental groups. However, the blood glucose concentrations of these rats were still above the range for normal rats and this does not make them hypoglycaemic. The reduction in blood glucose from the values obtained after induction of diabetes to that after treatment with the plant extract showed no significant ($p>0.05$) decrease with the exception of the rats in the group administered 500 mg/kg extract with induction of diabetes (Figure 1).

The haematological profile of rats in the experimental groups had higher PCV and haemoglobin concentrations which were significant compared with those in the diabetic control that received no treatment (see Table 3). Also, the plant extract was not only able to reduce the blood glucose levels of the rats which was found to be comparable to the effect observed with the standard drug, Glibenclamide.

Diabetes is characterized with increase in reactive oxygen species which are produced from the effect of alloxan and from the oxidation of the accumulated glucose via the TCA cycle and electron transfer in the mitochondria. More glucose is oxidized in diabetic cells via TCA, which in effect pushes more electron donors (NADH and $FADH_2$) into the electron transport chain. Complex III is blocked which happens when a threshold is reached causing electrons to back up to coenzyme Q, which donates the electrons one at a time to molecular oxygen, thereby generating superoxide radicals (Brownlee, 2005). Rani *et al.* (2010) reported that about 12 % of patients that are diagnosed with diabetes have some forms of anaemia that is associated with diabetes, and diabetes is characterized with oxidative stress.

Glucose accumulation in diabetes also results in the depletion of NADPH through the polyol pathway that eventually leads to sorbitol production (Bonnefont-Rousselot, 2002; Jay et al., 2006). Production of sorbitol is accompanied by a disproportionate use of reduced glutathione. This results in the decrease in cytosolic concentrations of reduced glutathione. NADPH is a crucial co-factor that plays roles in reductive processes, regeneration of oxidized glutathione that act in synergy with NADPH in the protection of red blood cells (Agarwal et al., 2013). Hyperglycaemia could ultimately lead to the destruction of the blood cells through the polyol pathway which could be one of the reasons why anaemia is associated with diabetes. Diabetes-related chronic hyperglycaemia can lead to a hypoxic environment in the renal interstitium, which could result in impaired production of erythropoietin by the peritubular fibroblasts and subsequently cause anaemia. Anemia in patients with diabetes mellitus might contribute to the pathogenesis and progression of cardiovascular disease and aggravate diabetic nephropathy and retinopathy (Singh et al., 2009).

Blood cells especially the RBCs are destroyed by a concert of events from the superoxide radicals produced in the electron transport chain in the mitochondrial with the decline in the availability of reduced glutathione and NADPH that are consumed in the polyol pathway and the resultant effect being anaemia. The haematinic properties of *A. heterophyllus* plant extract is supported with the high values in PCV, haemoglobin concentrations and red blood cell counts of the rats in the group that received no induction of diabetes but were fed the plant extract, of which similar increase were found in rats in the treatment groups.

Conclusion

From our findings in this study, that the ethanol leaf extract of *A. heterophyllus* increased the haematological indices of diabetic rats. The leaves of *A. heterophyllus* can also be used as a supplement in diabetic food to reduce the occurrence of anaemia.

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